

04-16-03

GAU-1645

AMENDMENT TRANSMITTAL LETTER (Small Entity)

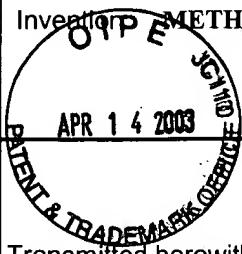
Applicant(s): SHABAHANG, Shahrokh et al.

Docket No.

13070-1

Serial No.
09/769,981Filing Date
January 25, 2001Examiner
HINES, Jana A.Group Art Unit
1645

Inventor(s) METHOD FOR THE EVALUATION OF IMPLANTABLE MATERIALS



RECEIVED

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TO THE ASSISTANT COMMISSIONER FOR PATENTS:

TECH CENTER 1600/2900

Transmitted herewith is an amendment in the above-identified application.

- Small Entity status of this application has been established under 37 CFR 1.27 by a verified statement previously submitted.
- A verified statement to establish Small Entity status under 37 FR 1.27 is enclosed.

The fee has been calculated and is transmitted as shown below.

CLAIMS AS AMENDED

	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST # PREV. PAID FOR	NUMBER EXTRA CLAIMS PRESENT	RATE	ADDITIONAL FEE
TOTAL CLAIMS	12 -	20 =	0	x \$9.00	\$0.00
INDEP. CLAIMS	1 -	3 =	0	x \$42.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT					\$0.00

- No additional fee is required for amendment.
- Please charge Deposit Account No. _____ in the amount of _____
A duplicate copy of this sheet is enclosed.
- A check in the amount of _____ to cover the filing fee is enclosed.
- The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 19-2090
A duplicate copy of this sheet is enclosed.
 - Any additional filing fees required under 37 C.F.R. 1.16.
 - Any patent application processing fees under 37 CFR 1.17.

Signature

David A. FARAH, M.D.

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Dated: April 14, 2003

I certify that this document and fee is being deposited on _____ with the U.S. Postal Service as first class mail under 37 C.F.R. 1.8 and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

EV247103065US

BY EXPRESS MAIL

Typed or Printed Name of Person Mailing Correspondence

cc: Loma Linda University



09/769,981

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:) Group Art Unit: 1645
SHABAHANG, Shahrokh et al.) Examiner: Jana A. Hines
Serial No.: 09/769,981)
Filed: January 25, 2001)
For: Method for the Evaluation of) Pasadena, California
Implantable Materials)

13070-1
#8/A #7/A
JAN
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RESPONSE AND AMENDMENT

Box Non-Fee Amendment
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the outstanding Office Action dated March 5, 2003, please amend the application as follows:

CERTIFICATE OF EXPRESS MAIL

"EXPRESS MAIL" mailing label number EV 247103065 US

Date of Deposit: April 14, 2003

I hereby certify that the attached papers are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Box Non-Fee Amendment, Commissioner for Patents, Washington, D.C. 20231.

Date: April 14, 2003

Marilyn C. Paik

Please amend the above-identified application as follows:

IN THE DISCLOSURE:

Page 8, lines 8-28:

Examples of the present method will now be described in greater detail. Modified bacteria containing genes to produce a functional green fluorescent protein, a functional luciferase and to contain an antibiotic resistance gene were constructed for use in the method by transformation with a plasmid DNA bearing a cassette with genes producing luciferase, green fluorescent protein and antibiotic resistance as follows. Two constructs were used. The first construct pLITE201 (as disclosed in Voisey CR, Marincs F. Biotechniques 1998; 24:56) was a plasmid vector with a gram-negative origin of replication containing the *lux CDABE* cassette from *Xenorhabdus luminescens* driven by the lac promoter. It was purified from DH5 α using the Maxi-Prep DNA purification kit (Qiagen GmbH, Santa Clarita, CA, US). The pLITE201 plasmid was then electroporated into attenuated strains of *Vibrio cholera*, *Salmonella typhimurium*, and *Shigella* using BIO-RAD® electroporation protocols for the various strains and the BIO-RAD® GENE PULSER® II unit (a pulse generator for transfection of nucleic acids into mammalian, plant, or bacterial cells, Bio-Rad laboratories, Hercules, CA). Positive transformants were identified by placing the outgrowth plates under the Argus 100 low light imager (Hamamatsu Corp., Hamamatsu, Japan). The positive colonies were confirmed by observing fluorescent bacteria under the fluorescent microscope.

The second construct was a *lux ABCDE* cassette from pXylA-dual (Hill, P, University of Nottingham, UK) as shown in Figure 1, purified using the Maxi-Prep kit (Qiagen). This plasmid has a gram-positive origin of replication as well as gram-positive ribosomal binding sites, which allowed expression in gram-positive organisms. The plasmid was then transformed into *Enterococcus faecalis* (strains JH2-2, ATCC4082 , and OG1X) using electroporation with the BIO-RAD® GENE PULSER® II (Bio-Rad Laboratories, Hercules, CA).